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Engineering plant abiotic stress tolerance by the overexpression of aldo/keto reductases

Zoltán Turóczy

Institute of Plant Biology, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary

Due to the sessile life style, plants are continuously exposed to a wide range of biotic and abiotic stress factors. This stress exposure severly affects their bioproductivity by causing the rapid and excessive accumulation of reactive oxygen species (ROS). ROS production in the vicinity of biomembranes containing polyunsaturated fatty acids can lead to lipid peroxidation and generate chemically reactive cleavage products, largely represented by aldehydes. Plant aldo/keto reductases (AKRs), among other enzymes, have been shown to be effective in the detoxification of lipid peroxidation-derived reactive aldehydes (Oberschall et al. 2000; Hideg et al. 2003).

In the present work we characterize a novel rice (*Oryza sativa*) AKR protein (OsAKR1) and investigate the transcriptomic changes in the gene expression profile of additional two AKR genes (*OsAKR2, OsAKR3*) in response to different stress treatments. A wide range of stress factors (abscisic acid, hydrogen-peroxide, mannitol etc.) was shown to trigger the expression of these AKR genes in rice cell suspensions, resulting in several folds of increased transcript levels. The most effective inducers were the ABA and hydrogen-peroxide, and *OsAKR1* gene turned out to be the most stress responsive. Stimulated by these results we investigated further the properties of the encoded protein by the *OsAKR1* gene, by cloning the full-length *OsAKR1* cDNA into recombinant protein expression construct, and purifying the glutathione-S-transferase (GST)-OsAKR1 fusion protein. Results of subsequent assays revealed that the GST-OsAKR1 recombinant protein exhibited a high, NADPH-dependent catalitic activity to metabolize toxic aldehydes (methylglyoxal, phenylglyoxal, glyoxal). Since cytotoxic reactive aldehydes can produce significant damages in the plant cells, the function of OsAKR1 protein to metabolize some of these harmful products was very promising. We also showed through *in vivo* experiments, that overproduction of this enzyme in *E. coli*, increased the tolerance of bacterial cells against high concentration (2mM) of methylglyoxal. The stress induced transcription of this AKR gene, as well as the data obtained from its biochemical characterization, supported its possible involvement in the abiotic stress induced reactive carbonyl detoxification pathways.

Till now there are several approaches to increase stress tolerance by manipulating the expression of endogenous, stress-related genes. Strategies targeting transcription factor expression have been shown to be effective, but on the other hand, stress tolerance can also be achieved by changing the expression of a single gene (Zhu 2001). Following the latter approach, we overexpressed the *OsAKR1* gene in tobacco (*Nicotiana tabacum*) and verified the effects of a single gene overexpression on the stress tolerance of the transgenic plants. We found, that the transgenic lines overproducing the OsAKR1 protein, accumulated significantly lower reactive aldehydes in response to the methylviologen (MV) treatment than the wild type. MV is a strong oxidative stress inducing herbicide, linked to ROS production and consequently to the formation of toxic aldehyde degradation products. In addition, the overexpressing lines reserved their photosynthetic functions more efficiently after heat treatments then the wild type. Therefore we suggest, that overexpression of a single gene (*OsAKR1*) and the accumulation of OsAKR1 protein is mainly beneficial in the detoxification processes against the reactive aldehydes generated at increased levels under stress conditions in the transgenic plants.

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Supervisor: Gábor V. Horváth
E-mail: placebo_tz@yahoo.com

Toxicogenomics screening of small molecules using high-density nanocapillary QRT-PCR technique

Laura Vass

Laboratory of Functional Genomics, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Hungary

Toxicogenomics combines studies of genomics, cell and tissue-wide protein expression and metabonomics to understand the role of gene-environment interactions in healthy and diseased samples. Predictive toxicogenomics is the acquisition of advanced knowledge of the safety profile of a compound using genomic biomarkers (Fielden et al. 2006). By clustering analysis of the gene expression profiles over selected biomarkers induced by the lead molecules and relevant derivatives, the medicinal chemist can deduce the relationship between structural modifications and changes in the toxicity profile (structure-toxicity relationship). Involvement of well-characterized reference compounds can be of help in this profiling, for instance defining the specific tissue or organ toxicity.